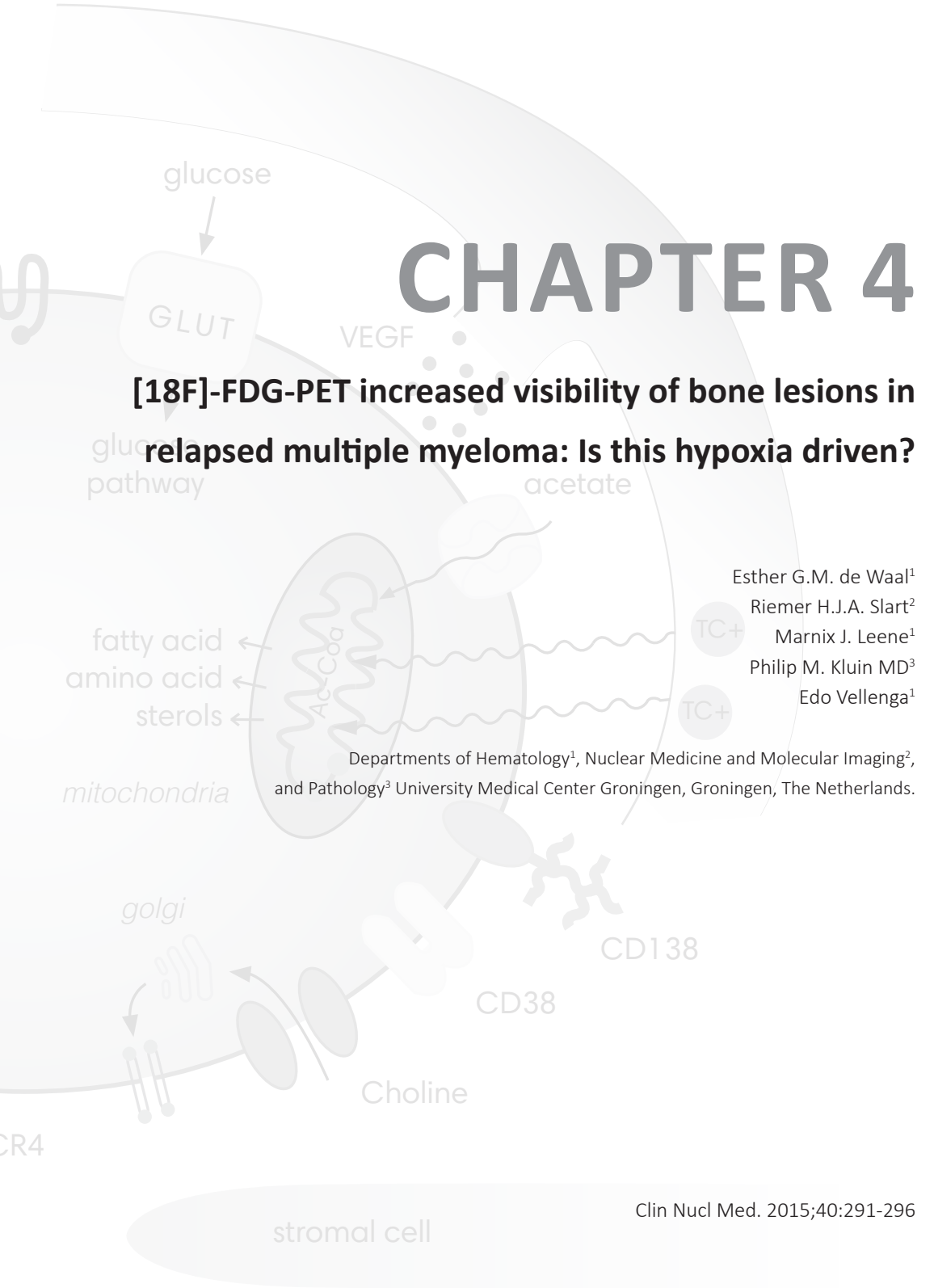
Multiple aspects of a plasma cell dyscrasia

de Waal, Elisabeth Geertruida Maria



CHAPTER 4

[18F]-FDG-PET increased visibility of bone lesions in relapsed multiple myeloma: Is this hypoxia driven?

Esther G.M. de Waal[1]
Riemer H.J.A. Slart[2]
Marnix J. Leene[1]
Philip M. Kluin MD[3]
Edo Vellenga[1]

Departments of Hematology[1], Nuclear Medicine and Molecular Imaging[2], and Pathology[3] University Medical Center Groningen, Groningen, The Netherlands.

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Abstract

Introduction: Whole body X-ray (WBX) is used for detecting skeleton abnormalities in multiple myeloma (MM) patients. An alternative might be [F-18]fluorodeoxyglucose positron emission tomography (FDG-PET) which make use of metabolic changes of malignant plasma cells. The aim of this study is to demonstrate whether [18F]-FDG-PET is more valuable in relapsing MM compared to WBX and to define its prognostic value. In addition 1-α-D: -(5-deoxy-5-[18F]-fluoroarabinofuranosyl)-2-nitroimidazole ([18F]-FAZA) scan and immunohistochemical staining on bone marrow was performed to define whether FDG uptake coincides angiogenesis related tumor hypoxia.

Methods: [18F]-FDG-PET (n=44) and [18F]-FAZA PET (n=5) was performed in patients with relapsed MM. Bone marrow biopsies (n=20) were evaluated for hypoxia inducible factors (HIF)-1α and-2α, vascular endothelial growth factor (VEGF), glucose transport protein (GLUT) 1 and 3 and the micro-vessel density (MVD).

Results: New lesions were more frequently demonstrated on [18F]-FDG-PET than on WBX (p=0.000001). [18F]-FDG-PET was not predictive for progression free survival and overall survival. Immunohistochemical staining on bone marrow biopsies demonstrated a significant increase in MVD and elevated expression of VEGF, HIF-2α and GLUT-3 by the malignant plasma cells. However HIF-1α expression and [18F]-FAZA scans were negative.

Conclusion: Our results demonstrate that [18F]-FDG-PET is relevant for diagnostic purposes compared to WBX in relapsing MM. The enhanced uptake of [18F]-FDG-PET is likely related to the activation of the HIF-2α signaling pathway, but probably independent of hypoxia induced signaling in view of the negative findings on both [18F]-FAZA PET and HIF-1α expression.

Keywords: relapsing multiple myeloma, [18F]-FDG-PET, [18F]-FAZA, immunohistochemical stainings, response to treatment.

Introduction

Multiple myeloma (MM) is a disease characterized by a monoclonal plasma cell population in bone marrow whereby osseous involvement is a predominant feature. In 90% of the patients' lytic bone lesions develop, which is an important cause of morbidity[1]. Lytic bone lesions are the result of increased bone resorption and reduced bone formation[2]. Whole body X-ray (WBX) is the method of choice for detecting skeleton abnormalities. This technique has several limitations such that it can detect only lesions that have lost more than 30% of the trabecular bone[3]. Furthermore the value in relapsing disease is limited because lesions persist after treatment so it cannot distinguish old vs. new active skeleton lesions. Therefore alternative techniques have been developed to visualize disease activity. 18-F-fluorodeoxyglucose positron emission tomography ([18F]-FDG-PET) uses the enhanced metabolic activity of cells to visualize the abnormal lesions[4]. In newly diagnosed MM patient [18F]-FDG-PET detects more lesions compared to WBX; furthermore, a positive [18F]-FDG-PET scan is of prognostic value[5]. This imaging technique may also be useful in patients with relapsing MM since it is not hampered by the presence of preexisting skeletal defects and visualizes areas of enhanced metabolic activity[6-8]. Indeed, in our previous study we demonstrated that [18F]-FDG-PET is a valuable tool for detecting MM activity in patients with relapsing MM[9]. In view of these encouraging results we extended the number of included patients having a [18F]-FDG-PET and a skeletal survey in the work up of relapsing MM to evaluate whether [18F]-FDG-PET indeed detects more lesions in patients with relapsing MM and has a prognostic value in the relapse setting of MM as well.

The enhanced metabolic activity defined by [18F]-FDG-PET could be related to hypoxia and the subsequent increased protein expression of hypoxia inducible factors (HIF) 1α and 2α, which can trigger the vascular endothelial growth factor (VEGF) pathway and stimulate angiogenesis[10,11]. Previous studies have shown that angiogenesis defined by increased micro-vessel density (MVD) is highly correlated with progressive disease in MM[12]. Recently new scanning methods have been developed for demonstrating *in vivo* tumor hypoxia. The PET tracer hypoxia, 1-α-D:-(5-deoxy-5-[18F]-fluoroarabinofuranosyl)-2-nitroimidazole ([18F]-FAZA), has been shown to accumulate in tumor hypoxia. In a study performed by Postema *et al.,* 50 patients with different types of malignancy (solid as well as hematologic), tumor hypoxia could be demonstrated in 46% of the patients[13].

Based on these findings we studied *in vitro* hypoxia related proteins including HIF 1α and 2α, VEGF and other hypoxia related proteins such as glucose transporter (GLUT) 1 and -3 expression on bone marrow biopsies of MM patients and determined MVD. In addition, *in vivo* [18F]-FAZA scans were performed in these patients to demonstrate whether tumor hypoxia can also be visualized *in vivo* in MM patients and whether this corresponds with elevated uptake of [18F]-FDG-PET.

Materials and Methods

Patients

A total of 44 MM patients were prospectively included in this study. Approval for the study was obtained from the local medical ethical committee. The diagnosis MM was based on the presence of a monoclonal plasma cell population in the bone marrow, the presence of a monoclonal immunoglobulin protein or elevation of free light chain (FLC) in serum (normal range FLC kappa 2.3 – 20.0 mg/l and FLC lambda 4.4 – 32.0 mg/l) and the presence of one or more of the following related organ or tissue impairments; lytic bone lesion, renal involvement, hypercalcemia or anemia. Relapse MM after having achieved CR was defined as follows: 1. reappearance of paraprotein; 2. more than 5% plasma cells in bone marrow; 3. new lytic bone lesions or progression of old lesions; 4. new hypercalcemia. Relapse MM after having achieved PR was defined as follows: 1. increase of paraprotein with more than 25%; 2. increase of urine paraprotein with more than 25%; 3. increase of plasma cells in bone marrow with 10%; 4. new lytic bone lesions or progression of old lesions; 5. new hypercalcemia, according to international guidelines[14].

In all patients with relapsing MM in our institute, a [18F]-FDG-PET scan was performed. A skeletal survey examination was performed in 38 patients. A subgroup of patients with a positive [18F]-FDG-PET scan result were randomly chosen to undergo an [18F]-FAZA. Patients were treated according to ongoing trials or protocols[15-17]. Of 44 patients, 37 (75%) were previously treated with autologous stem cell transplantation. Most of the patients were treated upfront with three cycles of induction with VAD (vincristine, doxorubicin and dexamethasone), TAD (thalidomide, doxorubicin and dexamethasone) or PAD (bortezomib, adriamycin and dexamethasone). This was followed by peripheral blood stem cell collection and transplantation. Treatment schedules used at relapse included bortezomib, lenalidomide, thalidomide, dexamethasone and in some cases in combination with cyclophosphamide or melphalan. At last follow up 26 of the 44 patients are alive.

Response to treatment was measured using the standard criteria, although not all patients underwent a repeated bone marrow biopsy to establish complete response. CR is defined as no m-protein or FLC present and normal bone marrow, very good partial response (VGPR) is defined as more than 90% reduction in m-protein or FLC. PR is defined as a more than 50% reduction in m-protein or FLC and progressive disease as an increase in m-protein or FLC with more than 25%[14].

Immunohistochemistry for MVD, VEGF-A, HIF 1α and 2α and GLUT-1 and -3

The expression of angiogenesis and hypoxia related features was examined by immunohistochemical staining on bone marrow biopsies (n=20). After fixation in 10% neutral phosphate buffered formalin (3.6% formaldehyde) for at least 12h, decalcification in a solution

containing 10% (v/v) acetic acid and 10% formalin (v/v; 3.6% formaldehyde) for 1 or 2 days. Than the bone marrow was paraffin embedded and cut in sections of 4 µm and stained as follows. Slides were dewaxed and rehydrated in graded alcohol solutions. Endogenous peroxidase activity was blocked with hydrogen peroxide. Antigen retrieval was done heat-induced with the microwave, expect for the VEGF staining were antigen retrieval was done with protease 0.1%. Slides were than incubated with the primary antibody for GLUT-1 (rabbit polyclonal 07-1401, Millipore USA; diluted 1:750), GLUT-3 (rabbit polyclonal ab15311, Abcam UK; diluted 1:100), HIF-1α (mouse monoclonal clone 54/HIF-1α BD Biosciences; diluted 1:70) HIF-2α (mouse monoclonal ab8365, Abcam; diluted 1:200) and VEGF-A (rabbit polyclonal sc-152, Santa Cruz USA; diluted 1:50). Blood vessels were visualized with a CD34+ antibody (mouse monoclonal QBEnd 10, Dako, Copenhagen, Denmark). The slides were washed with PBS and after that incubated with a rabbit anti mouse or goat anti rabbit horse radish peroxidase conjugated as secondary or tertiary antibody. For the VEGF staining streptavidine peroxidase was used as a tertiary antibody. The chromogenic reaction was performed with diaminobenzidine for 12 min and after that sections were counterstained with haematoxylin. Finally, the slides were dehydrated and mounted.

The intensity of staining was analyzed semi-quantitatively. The percentage of positive plasma cells was counted according the following scoring system: no visibility, 10-30% positive, 30-50% positive, 50-80% positive and more than 80% positive plasma cells[18]. The vessel count was measured using light microscopy in areas of the slide containing highest numbers of blood vessels per selected area (hotspot). After the hotspots were identified, the total number of vessels per selected image was counted at 400 magnifications. At least five fields were counted for each section and the true vessel number was expressed as the mean of 5 counts[19].

Skeletal survey

Each patient underwent a whole body X-ray survey, consisting of X-skull, X-humeri, X-femora, X-whole spine, X-pelvis. Conventional radiographs were acquired at 73 kVp and 16 mAs by using a radiographic imager (MULTIX Swing; Siemens Medical Systems, Chicago, Ill) by using a bucky grid.

[18F]-FDG-PET

All patients had to fast for at least 5 h before undergoing [18F]-FDG-PET. FDG was administered intravenously (4-5 MBq/kg). After an uptake period of 60 min, PET emission data were acquired from total body, 5 min per bed position. A Biograph mCT scanner was used (Siemens Medical Systems, Knoxville, TN, USA). The measured resolution is 2-4 mm in full width at half maximum transaxially in the center of the field of view. For PET data reconstruction 3 interactions, 21 subsets, with an image size of 256 x 256, zoom 1, were used.

FDG uptake was quantified using the maximal standardized uptake value (SUVmax) analysis in all patients. A standardized protocol for quantification of SUVmax was used, including blood glucose correction[20]. The region of interests used in SUV analysis were 3-dimensional and were based on the max value within the 50% isocontour boundaries using a Siemens workstation (Siemens Medical Systems, Knoxville, TN, USA).
The number, size, localization and SUVmax of focal lesions were recorded. The [18F]-FDG-PET scan was either negative with no focal lesions or positive with focal lesions. In addition, definition by Zamagni et al was used, defining PET positivity as more than 3 lesions or SUVmax > 4.2[5].
The skeletal survey and [18F]-FDG-PET were performed within a month of each other. [18F]-FDG-PET images were examined by an experienced nuclear physician using also visual examination with special attention to the skeleton.

[18F]-FAZA

Procedures for good-manufacturing-practice production of the hypoxia tracer [18F]-FAZA have been developed previously. The synthesis of [18F]-FAZA was optimized using a Micro Fluid Chemistry Module (Advion). The routine production was performed using a robot system (Zymark). Briefly, the precursor (2 nitro imidazole) for labeling [18F]-FAZA was reacted with dried 18F/K222 complex in dimethyl sulfoxide and thereafter deprotected with 0.1 M NaOH. After high-performance liquid chromatography purification of the reaction mixture, $_{[18F]-}$FAZA was formulated using an Oasis HLB plus cartridge. The final sterile solution was analyzed using high-performance liquid chromatography and released for administration to the patient.
[18F]-FAZA PET scans were performed on the same mCT camera as the [18F]-FDG images according to local standard operating procedures for [18F]-FAZA PET scans. Patients were injected with 370 MBq intravenously. After a waiting period of 120 min, a scan was obtained from the midthigh to the brain and analyzed using the previous-mentioned research workstation. [18F]-FAZA SUVmax was quantified in the same way as [18F]-FDG SUVmax, including correction for the partial-volume effect. The median time interval between [18F]-FDG PET and [18F]-FAZA PET was 21 days (range 1-51). The data were reconstructed with time-of-flight, high-definition, ordered-subsets expectation maximization using 3 iterations, 21 subsets, and a 5-mm gaussian postprocessing filter and had a spatial resolution of 2.04 × 2.04 × 2 mm.

Statistical analysis

Difference between the amount of lesions between WBX and [18F]-FDG-PET were calculated from the data using a Wilcoxon signed ranks test. Survival was calculated using the method of Kaplan and Meier and compared by log rank test. Correlation was calculated using a Spearmans rho. $P < 0.05$ was considered significant.

Results

This study included 44 patients. The group consisted of 27 men and 17 women with a median age of 62.5 years (range 48 to 78 years). Median number of prior treatment regimens was 3.5 (range 2-12). Patient characteristics are presented in table 1. Fifty-five percent of patients showed production of monoclonal IgG, 8% of IgA and 35% patients only showed production of serum FLC.

Table 1: Patient characteristics

Characteristic (n = 44)	Number	%
Age (years)		
median	62	
range	48 – 78	
gender		
male	27	61
female	17	39
no of prior treatment regimens		
median	3.5	
range	02 – 12	
Type of m-protein		
IgG	27	55
IgA	4	8
IgM	0	0
FLC only	17	35
% plasma cells bone marrow at relapse MM		
No bone marrow	4	10
1-Oct	22	45
Oct-30	17	35
> 30	5	10
Type of prior treatment		
Autologous stem cell transplantation	42	86
Allogeneic stem cell transplantation	6	12
Lenalidomide	32	65
Bortezomib	43	78
Thalidomide	38	76
Low dose melphalan	14	29

Legend: FLC; free light chain. MM; multiple myeloma.

During the follow up 5 patients experienced two episodes of relapse. For each relapse [18F]-FDG-PET was performed. Not in all cases a skeletal survey was performed in combination with [18F]-FDG-PET. In total 49 [18F]-FDG-PET scans were performed and 38 skeletal survey examinations. In 79% of the patients pre-existing defects were demonstrated on the skeletal survey. New abnormalities at relapse were demonstrated in 42% of the patients. The median

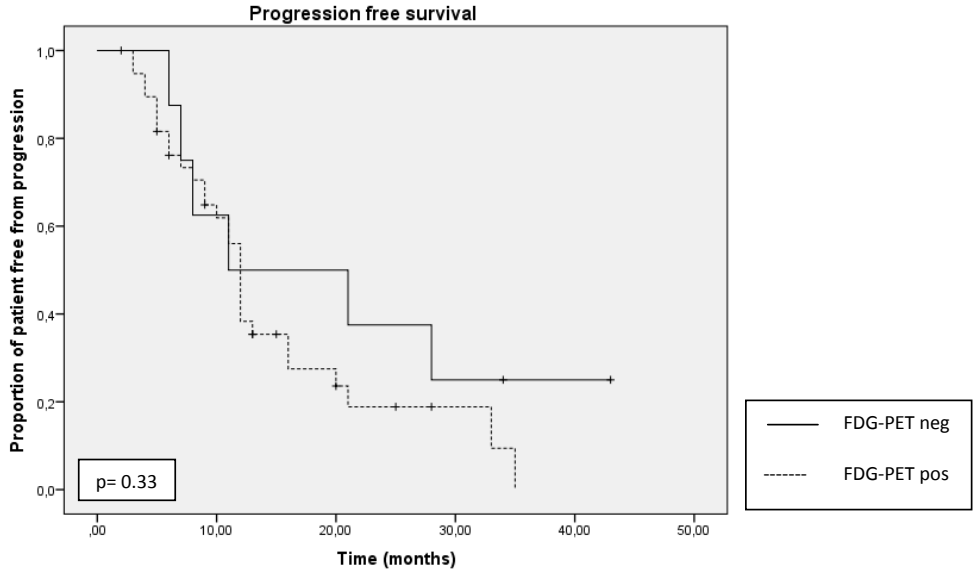
number of skeletal lesions on X-ray was 0 (range 0-7). The [18F]-FDG-PET showed abnormal uptake in 82% of the patients. No diffuse increased FDG uptake in bone marrow was noticed in the studied patients. The median number of FDG lesions was 4 (range 0-22). The average SUVmax was 5.4 with a highest SUVmax of 14.1 and a lowest SUVmax of 1.7. The number of lesions was significantly higher with the [18F]-FDG-PET vs. skeletal survey (p = 0.000001). One patient had a lesion in the skull, not detected on [18F]-FDG-PET. One other patient had several small lesions (1 cm or smaller) in pelvic and femur also not detected with [18F]-FDG-PET. None of the patients received treatment before the skeletal survey and [18F]-FDG-PET. All other lesions defined by the skeletal survey were confirmed on the [18F]-FDG-PET. In addition, in 5 patients extra-osseous lesions were detected with strong positive FDG avid lesions (median SUVmax 9.7). None of these lesions were demonstrated on plain X-ray. For 9 patients no abnormal lesions could be demonstrated on both [18F]-FDG-PET and WBX although all patients had distinct signs of relapsing disease.

Treatment results were available for all 44 patients. CR was obtained in 13 (27%) of the patients, VGPR in 6 (12%) patients, PR in 13 (27%) patients and progressive disease in 13 (27%) patients. For 3 patients it was not possible to measure treatment response, 1 patient was lost during follow up, 1 patient died due to another cause and for 1 patient the follow up interval was too short. Median follow-up was 14 months (range 2-49). Median progression free survival (PFS) was 11.7 months (range 2-43). For patients reaching a CR the median PFS was 16 months compared to 5.3 months for patients with progressive disease (p= 0.00001). Overall survival was not reached for patients with CR vs. 8 months for patients with progressive disease (p = 0.000001).

In 12 patients [18F]-FDG-PET was performed after chemotherapy treatment. Nine patients had responsive disease with decrease in the number of lesions according to the [18F]-FDG-PET. Of 9 patients, 3 (33%) had a CR on the [18F]-FDG-PET. Two patients had stable disease and 1 patient had progressive disease. On the [18F]-FDG-PET before treatment there were median 7.5 lesion (range 1-22) and after treatment 3 lesions (range 0-10). The median SUV max declined from 5.5 (range 3.3-9.4) to 2.27 (range 0-4.6) (p=0.003). Response observed with [18F]-FDG-PET was comparable to clinical response.

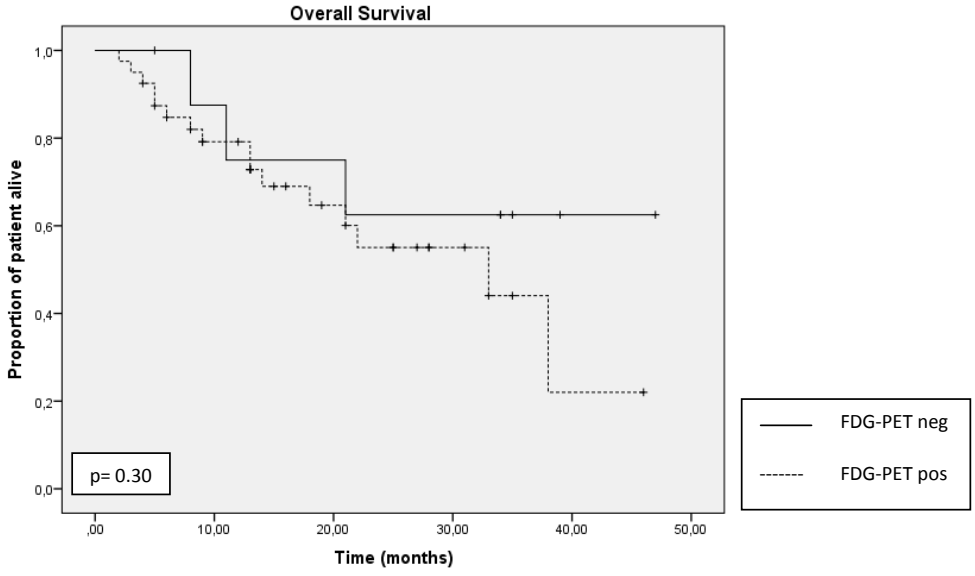
Overall, [18F]-FDG-PET findings were not predictive for PFS (p =0.30) and OS (p=0.33) and also when PET positivity was defined as more than 3 lesions or SUVmax > 4.2[5]. Figure 1A and B show the PFS and overall survival (OS) related to [18F]-FDG-PET outcome.

Figure 1A: Progression free survival correlated to FDG-PET outcome



Legend: The correlation between [18F]-[18F]-FDG-PET outcome and progression free survival.

Figure 1B: Overall survival correlated to FDG-PET outcome



Legend: The correlation between [18F]-[18F]-FDG-PET outcome and overall survival.

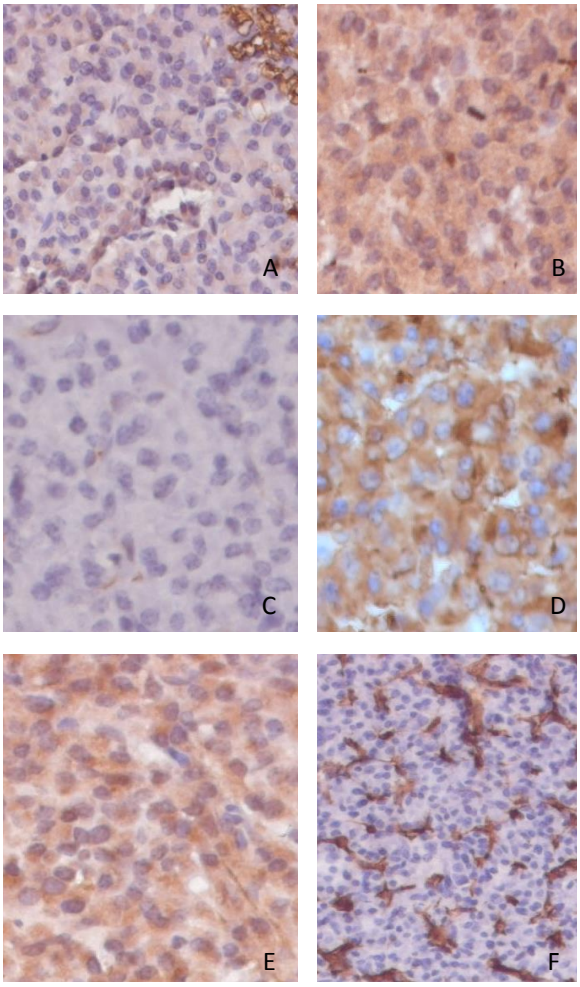
Immunohistochemical staining on bone marrow biopsies (n=20) with the CD34 monoclonal antibody demonstrated an increased MVD scoring of median 13.5 (range 3-200) vs. 3.5 on normal bone marrow[21]. The MVD was particular high when clusters of plasma cells were present. In 80% of the bone marrow biopsies more than 50% of the plasma cells were positive for VEGF-A. The staining for the GLUT demonstrated that 10% of the biopsies showed more than 50% of the plasma cells positive for GLUT-1 and in 55% of the biopsies for GLUT-3. A clear distinction was observed for HIF-1α and HIF-2α; no staining for HIF-1α was demonstrated on plasma cells although the surrounding endothelial cells demonstrated a distinct positive staining, while in 85% of the samples more than 50% of the plasma cells were positive for HIF-2α. Table 2 shows the results of immunohistochemistry combined with the results of the [18F]-FDG-PET and treatment outcome. Figure 2 provides the results of the staining from 1 patient. MVD was the only factor that correlated with [18F]-FDG-PET positivity ($p = 0.03$). The expression of VEGF ($p = 0.67$), HIF-2α ($p=0.33$) or GLUT-3 ($p =0.50$) was not predictive for having a positive or negative [18F]-FDG-PET result.

Table 2: Findings of immunohistochemical staining, [18F]-FDG-PET and response to treatment

Nr	VEGF	MVD	GLUT-1	GLUT-3	HIF-1α	HIF-2α	FDG-PET	FDG-PET > 3	SUV-MAX	Respons to treatment
1	>80%	5	50-80%	10-30%	neg	50-80%	pos	pos	14,5	progr disease
2	>80%	3	neg	>80%	neg	>80%	neg	neg	0	CR
3	>80%	3	neg	neg	neg	50-80%	pos	pos	13,2	PR
4	>80%	14	neg	neg	neg	50-80%	pos	neg	3,7	CR
5	>80%	85	neg	neg	neg	30-50%	pos	pos	6,4	PR
6	30-50%	4	neg	neg	neg	10-30%	pos	pos	4,5	CR
7	>80%	200	10-30%	>80%	neg	>80%	pos	pos	4,3	CR
8	50-80%	4	50-80%	999	neg	50-80%	neg	neg	0	-
9	30-50%	13	neg	50-80%	neg	>80%	pos	pos	8	PR
10	>80%	6	10-30%	50-80%	neg	>80%	neg	neg	0	progr disease
11	50-80%	23	neg	neg	neg	>80%	pos	pos	8,4	progr disease
12	50-80%	12	neg	50-80%	neg	>80%	pos	pos	22,2	progr disease
13	>80%	155	neg	>80%	neg	>80%	pos	pos	3,2	PR
14	>80%	86	30-50%	>80%	neg	>80%	pos	neg	4,6	PR
15	>80%	26	10-30%	>80%	neg	50-80%	pos	pos	5,6	VGPR
16	>80%	72	10-30%	>80%	neg	>80%	pos	pos	22,2	progr disease
17	10-30%	25	neg	10-30%	neg	50-80%	pos	neg	5,3	PR
18	10-30%	4	neg	10-30%	neg	30-50%	pos	neg	12,3	†
19	>80%	40	neg	>80%	neg	>80%	pos	pos	6,5	VGPR
20	50-80%	5	neg	50-80%	neg	>80%	neg	neg	0	SD

Legend: The findings of immunohistochemical staining, FDG-PET and response to treatment. GLUT; glucose transporter, HIF; hypoxia inducible factors, VEGF; vascular endothelial growth factor, MVD; micro-vessel density, FDG-PET>3: more than 3 lesions found on the FDG-PET as defined by Zamagni [5]. SUV-max; maximal standardized uptake value, CR; complete response, VGPR; very good partial response, PR; partial response, SD; stable disease, progr disease; progressive disease. † Died of other cause. Patient number 8 lost to follow up.

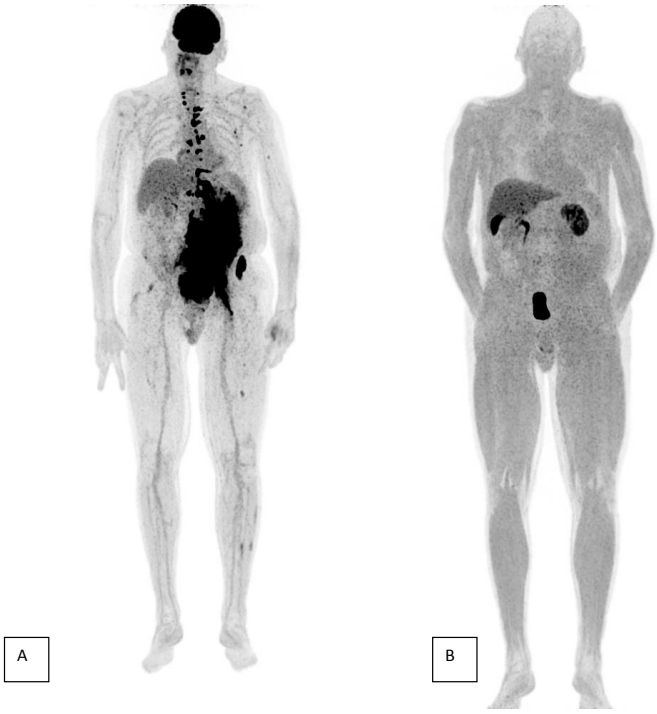
Figure 2: Immunohistochemical staining for GLUT-1 and 3, HIF-1α and HIF-2α, VEGF and MVD



Legend: Immunohistochemical staining for A:GLUT-1, B:GLUT-3, C:HIF-1α, D:HIF-2α, E:VEGF and F:MVD. GLUT; glucose transporter, HIF; hypoxia inducible factors, VEGF; vascular endothelial growth factor, MVD; micro-vessel density.

Five randomly chosen patients (4 male and 1 female) underwent an [18F]-FAZA PET scan. All five patients had a positive [18F]-FDG-PET scan. They received no treatment before the [18F]-FAZA scan. They had a median of 0 (range 0-1) lesions on the WBX and a median of 7 (range 2-15) lesions on the [18F]-FDG-PET. The median SUVmax was 9.4 (range 4.2-13.6). No lesions were demonstrated on [18F]-FAZA PET. Figure 3 demonstrates a patient with clear [18F]-FDG-PET positivity of a large extramedullary lesion but without uptake on [18F]-FAZA PET.

Figure 3: [18F]-FDG-PET and [18F]-FAZA of the same patient with relapsing MM



Legend: [18F]-FDG-PET and [18F]-FAZA image of patient (number 16 in table 2) with progression of MM. Large extramedullary and multiple ossal lesions are visible on the [18F]-FDG-PET scan (left scan). No lesions were visible on the [18F]-FAZA scan (right scan).

Discussion

Skeletal survey is the standard method for the detection of skeletal lesions in patients with MM. In relapsing disease measurement of disease progression is difficult to assess with this method because of the presence of pre-existing skeleton abnormalities. New techniques are therefore explored to demonstrate disease progression. In particular, MRI scanning is recommended for detecting osteolytic lesions in the spinal and pelvic regions. The disadvantage of this technique is the difficulty to use it for response evaluation since it takes 9-12 months before lesions resolve on MRI[8]. In addition, it has limitations for detecting bone marrow infiltration in ribs, clavicle and skull[3].

In the present study we used the property of enhanced metabolic activity shown by increased glucose uptake by the malignant plasma cells visualized on [18F]-FDG-PET. Thus far studies with [18F]-FDG-PET have been performed in untreated MM patients, demonstrating more positive lesions on the [18F]-FDG-PET scan in comparison to WBX[3]. The present study in

patients with relapsing MM also demonstrated a higher number of lesions with FDG-PET as compared with WBX. This is probably due to the fact that over 30% of the trabecular bone must be lost before osteolytic lesions are visible on WBX[3].
In general the lesions found on WBX were also demonstrated with [18F]-FDG-PET, except for small, diffuse defects. It has been demonstrated that small lesions, < 1 cm, are difficult to detect with [18F]-FDG-PET[23], due to limited resolution of the PET camera. We observed new skeletal survey lesions in 42% of the patients. This number might overestimate the real number of skeletal lesions after the last relapse because a number of patients had several relapses and skeletal survey was not performed on every occasion. On the other hand, extramedullary MM will be missed on WBX and can be appropriately diagnosed with [18F]-FDG-PET[4].
[18F]-FDG-PET has become an established imaging modality for the detection of osseous lesions in newly diagnosed MM patients and seems to be an independent prognostic parameter for overall survival[5]. In relapsing MM patients only limited studies have been performed. Derlin *et al*, demonstrated in relapsing MM patients following allogeneic stem cell transplantation a positive [18F]-FDG-PET scan result in 80% of the patients[24], which is comparable to our patients following autologous stem cell transplantation. Overall, we noticed that [18F]-FDG-PET provides more information than does WBX for detecting skeleton lesions in relapsing MM patients.
[18F]-FDG-PET has prognostic value for the treatment results in newly diagnosed MM patients[5]. However, in this cohort of relapsing MM we could not demonstrate the prognostic significance for [18F]-FDG-PET positivity. This might be due to the great variability in the number of relapses and prior treatment regimens. Median PFS was 11.7 months (range 2-43), which is comparable to other studies in patients with relapsing disease[25]. The most important factor was the response to treatment, patients reaching CR having a better PFS and OS (p=0.00001).

To study in greater detail the cause of the enhanced uptake of [18F]-FDG-PET in MM, we studied different parameters that are linked and may influence FDG uptake activity, such as tumor hypoxia. The PET tracer [18F]-FAZA makes use of this condition. Two-nitroimidazole compounds undergo reduction under hypoxic conditions, forming highly reactive oxygen radicals. This binds to macro-molecules inside the cell. When labeled with a radioisotope, this can be visualized with a PET-scanner. [18F]-FAZA has been used in patients with solid tumor like head and neck cancers and non-small cell lung cancers to detect tumor hypoxia[26, 27]. [18F]-FAZA scan was positive in two third of the head and neck cancer patients and in all non-small cell lung cancer patients[26, 27]. In our study we evaluated the regional uptake of [18F]-FAZA in patients with relapsing MM with a positive [18F]-FDG-PET scan result. However, in none of the 5 patients an enhanced uptake of the [18F]-FAZA could be demonstrated.

It is well known that bone marrow of MM patients reveal higher MVD and increased VEGF expression[12]. The expression of VEGF can be regulated by hypoxia inducible factors like HIF-1α and HIF-2α. In contrast to the study of Giatromanolaki et al[11] who demonstrated that 33% of the MM patients stained positive for HIF-1α, no HIF-1α staining of plasma cells was demonstrated in our patients. In addition we observed a low GLUT-1 expression, which is in line with the results of the HIF-1α staining since GLUT-1 is a downstream target of HIF-1α. The negative [18F]-FAZA scan result corresponds also with these findings. However HIF-2α expression was demonstrated in a high number of samples in conjunction with an increase in MVD and elevated expression of VEGF, suggesting that the HIF signaling pathway was activated. The number of MVD also correlated with the positivity on [18F]-FDG-PET. In solid tumor different expression patterns of HIF-1α and HIF-2α are described, suggesting different roles for both factors. It has been proposed that HIF-1α increases directly in response to hypoxia, while HIF-2α is responsive during prolonged period of hypoxia[28]. However, expression of HIF or VEGF is not synonymous with tumor hypoxia. Alternative pathways can be activated due to oncogene activation or mutations that trigger the expression of HIF. For example, it has been shown that signal transducer and activator of transcription 5 (STAT-5) can activate the expression of HIF-2α. STAT-5 can be activated by several cytokines and it induces expression of several target genes, including HIF-2α[29]. Whether STAT-5 plays an important role in multiple myeloma is not extensively studied but this could explain the observed difference in uptake between [18F]-FDG-PET and [18F]-FAZA.

In summary, our results demonstrate that in relapsing MM patients [18F]-FDG-PET is highly relevant for diagnostic purposes compared with WBX, but has limited value as prognostic parameter. The enhanced uptake of [18F]-FDG-PET is likely related to the activation of the HIF signaling pathway but not related to hypoxia in view of the negative findings on [18F]-FAZA PET.

Conflict of interest statement: none

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SST
Methionine
Tyrosine
LAT1
protein
synthesis
FLT
TK
Oxygen
radical
nucleus
nitroimidazole
Hypoxia
VLA
VCAM
CXC